

1 Claims

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3 1. A protein interaction system comprising

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5 a plurality of bait fusion proteins, each
6 fusion protein comprising (i) a first fragment
7 of fluorescent protein, a first peptide of
8 interest and a linker portion interposed
9 between the first peptide and first fluorescent
10 fragment; wherein the linker portions of each
11 bait fusion protein are of different lengths,
12 and the first peptide of interest of each bait
13 fusion protein is identical to the first
14 peptide of interest in each of the other bait
15 fusion proteins,

16

17 and (ii) at least one prey fusion protein
18 comprising a fragment of fluorescent protein
19 complementary to said first fragment of
20 fluorescent protein, a second peptide of
21 interest and a second linker portion interposed
22 between the complementary fragment and the
23 second peptide;

24

25 wherein, on interaction of a first peptide of
26 interest with a second peptide of interest,
27 the fragments of the fluorescent protein
28 functionally associate to promote
29 fluorescence.

30

31 2. The protein interaction system as claimed in
32 claim 1 or claim 2 wherein the linker

1 portions comprise in the range 5 to 100
2 amino acid residues.

3

4 3. The protein interaction system as claimed in
5 claim 2 wherein at least one linker portion
6 comprises at least 20 amino acids.

7

8 4. The protein interaction system according to
9 any one of claims 1 to 3, wherein the
10 fragments of fluorescent protein are
11 generatable through the introduction of a
12 split point between the amino acids at
13 positions 157 and 158, or (in a second
14 embodiment) between the amino acids at
15 positions 172 and 173 of the humanised form
16 of Green Fluorescent Protein (SEQ ID NO 2).

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18 5. The protein interaction system as claimed in
19 any one of the preceding claims, wherein the
20 system comprises a plurality of prey fusion
21 proteins.

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23 6. The protein interaction system as claimed in
24 claim 5 wherein the linker portions of at
25 least two prey fusion proteins are of
26 different lengths.

27

28 7. The protein interaction system as claimed in
29 claim 5 or 6 wherein at least two of the
30 second peptides of interest of the prey
31 fusion proteins are provided by different

1 amino acid sequences.

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3 8. The protein interaction system as claimed in
4 any one of the preceding claims wherein the
5 first peptide is linked to the N terminus of
6 the first fragment of fluorescent protein.

7

8 9. The protein interaction system as claimed in
9 any one of claims 1 to 7 wherein the first
10 peptide is linked to the C terminus of the
11 first fragment of fluorescent protein.

12

13 10. The protein interaction system as claimed in
14 any one of the preceding claims wherein the
15 second peptide is linked to the N terminus
16 of the complementary fragment of fluorescent
17 protein.

18

19 11. The protein interaction system as claimed in
20 any one of claims 1 to 9 wherein the second
21 peptide is linked to the C terminus of the
22 complementary fragment of fluorescent
23 protein.

24

25 12. The protein interaction system as claimed in
26 any one of the preceding claims further
27 comprising at least a third fusion protein
28 comprising at least a third fragment of
29 fluorescent protein complementary to a first
30 and / or second complementary fragment of
31 fluorescent protein;
32 wherein said at least third fragment is

1 linked to at least a third peptide of
2 interest and at least a third linker is
3 interposed between the at least third
4 fragment and at least third peptide of
5 interest wherein the at least third fragment
6 of fluorescent protein is capable of
7 functional association with a first and / or
8 complementary fragment of fluorescent
9 protein such that on functional association
10 of said fragments fluorescence is enabled
11 and on interaction of the first, second and
12 third peptides of interest the fragments
13 functionally complement each other to
14 promote fluorescence.

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16 13. A protein interaction system as claimed in
17 any one of the preceding claims wherein the
18 system is a cell based system.

19

20 14. A library of nucleic acid constructs
21 comprising a plurality of nucleic acid
22 constructs, each construct encoding
23 (i) a first fragment of fluorescent protein
24 capable of functional association with a
25 complementary fragment of fluorescent
26 protein such that on functional association
27 of said first and complementary fragments
28 fluorescence is enabled,
29 (ii) a peptide of interest and
30 (iii) a linker portion interposed between
31 the peptide and first fragment of
32 fluorescent protein; wherein the peptide of

- 1 interest encoded by each nucleic acid
2 construct is the same and the linker portion
3 encoded by each construct is of a different
4 length to the linker encoded by each other
5 construct.
6
- 7 15. The library according to claim 14, wherein
8 the linker portions comprise in the range 5
9 to 100 amino acid residues.
10
- 11 16. The library as claimed in claim 14 or claim
12 15 wherein at least one linker portion
13 comprises at least 20 amino acids.
14
- 15 17. The library according to any one of claims
16 14 to 16, wherein the fragments of
17 fluorescent protein are generatable through
18 the introduction of a split point between
19 the amino acids at positions 157 and 158, or
20 (in a second embodiment) between the amino
21 acids at positions 172 and 173 of the
22 humanised form of Green Fluorescent Protein
23 (SEQ ID NO 2).
24
- 25 18. An expression vector comprising at least one
26 of the plurality of nucleic acid constructs
27 as defined in claims 14 to 17, wherein the
28 at least one nucleic acid construct encodes
29 a fusion protein having a linker of at least
30 20 amino acids.
31

- 1 19. An expression vector comprising a plurality
2 of nucleic acid constructs as defined in any
3 one of claims 14 to 17.
4
- 5 20. The expression vector according to claim 19,
6 wherein at least one nucleic acid construct
7 encodes a fusion protein having a linker of
8 at least 20 amino acids.
9
- 10 21. A cell transformed with a vector as claimed
11 in any one of claims 18 to 20.
12
- 13 22. A cell comprising a protein interaction
14 system as claimed in any one of claims 1 to
15 13.
16
- 17 23. The cell according to claim 22, wherein the
18 cell is a cell according to claim 21.
19
- 20 24. An assay method for monitoring peptide
21 interaction comprising the steps of
22 (i) providing the protein interaction system
23 of any one of claims 1 to 13;
24 (ii) allowing the bait fusion proteins to
25 come into contact with the prey fusion
26 protein(s): and
27 (iii) measuring fluorescence produced by the
28 interaction of a first and second peptide of
29 interest causing fragments of the
30 fluorescent protein to functionally
31 interact.
32

- 1 25. The assay method according to claim 24,
2 wherein the assay is a cell-based assay.
3
- 4 26. The assay method according to claim 25,
5 wherein the cell based assay is performed
6 using one or more cells according to claim
7 22 or claim 23.
8
- 9 27. The method according to claim 25 or claim
10 26, wherein the assay further comprises the
11 step of determining the subcellular location
12 of the interaction of the first and second
13 peptides of interest in the at least one
14 cell.
15
- 16 28. The method according to any one of claims 24
17 to 27, wherein the assay further comprises
18 the step of determining the length of the
19 linker(s) of those fusion proteins which
20 allow the first fragment and complementary
21 fragment of the fluorescent protein to
22 functionally complement each other and
23 enable fluorescence to be detected on
24 interaction of the first and second peptide
25 of interest.
26
- 27 29. The method according to any one of claims 24
28 to 28, wherein the assay comprises the steps
29 of:
30 providing a putative interaction modulating
31 agent;
32 measuring the fluorescence produced in the

1 presence of said putative modulating agent;
2 comparing the measured fluorescence in the
3 presence of the putative modulating agent
4 with the measured fluorescence in the
5 absence of the putative modulating agent;
6 wherein a decrease in detection of
7 fluorescence in the presence of the putative
8 modulating agent relative to in the absence
9 of the putative modulating agent is
10 indicative that the putative modulating
11 agent prevents or is an inhibitor of peptide
12 interaction; and wherein an increase in
13 detection of fluorescence in the presence of
14 the putative modulating agent relative to in
15 the absence of the putative modulating agent
16 is indicative that the putative modulating
17 agent promotes or enhances peptide
18 interaction.

19
20 30. A kit comprising a library of nucleic acid
21 constructs according to any one of claims 14
22 to 17 and means to express the constructs.

23
24 31. The kit according to claim 30 which further
25 includes at least one second nucleic acid
26 construct which encodes a complementary
27 fragment of fluorescent protein, a second
28 peptide of interest and a second linker
29 portion interposed between the complementary
30 fragment and the second peptide of interest.

31

1 32. The kit as claimed in claim 31 wherein the
2 kit comprises a plurality of second nucleic
3 acid constructs, wherein the second peptides
4 of interest encoded by the plurality of
5 second nucleic acid constructs are each of
6 different amino acid sequence.

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